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Abstract

In this work, a series of novel low melting mixtures (LMM) based on cyclodextrins (CD) and levulinic acid and inspired by the deep eutectic solvents (DES), were prepared. These supramolecular mixtures are the first reported CD-based mixtures that are liquid at room temperature. Density, viscosity and rheological measurements as well as differential scanning calorimetry and thermogravimetric analysis were performed to characterize these new LMM. Nuclear magnetic resonance (NMR) spectroscopy was used to monitor their stability. Furthermore, their ability to solubilize *trans*-anethole (AN) and related essentials oils were evaluated by static headspace-gas chromatography (SH-GC), in comparison with water. AN was up to 1300 times more soluble in the CD-based LMM than in water. Finally, multiple headspace extraction (MHE) was used to monitor the release of AN from these LMM. After 10 extractions, 20 to 40% of AN was released from the studied LMM, while 70% was released from water. The new CD-based LMM have potential applications for solubilization and delivery of poorly soluble drugs.

Keywords: cyclodextrins, static headspace-gas chromatography, *trans*-anethole, essential oils, low melting mixtures.

1. Introduction

Cyclodextrins (CD) are non-toxic cyclic oligosaccharides obtained from the enzymatic degradation of starch (Crini, 2014; Szejtli, 1998). Native CD, which are produced at an industrial scale, consist of six (α-CD), seven (β-CD) or eight (γ-CD) α-(1→4) linked D-glucopyranose units. Due to their hydrophobic internal cavity and hydrophilic external surface, these water-soluble molecules have been extensively used to encapsulate hydrophobic and volatile compounds by forming host-guest inclusion complexes (Ciobanu et al., 2013; Kfoury et al., 2019, 2015; Marques, 2010).

β-CD is the most studied and most frequently used CD, because of its cheapness and availability. However, it is the least water-soluble of the three native CD. Therefore, various β-CD derivatives have been synthetized and are commercially available. CD and their derivatives have attracted interest for a wide range of applications such as food, agrochemistry, environmental chemistry, catalysis, cosmetics and pharmaceutics (Crini et al., 2018; Kurkov and Loftsson, 2013; Landy et al., 2012; Legrand et al., 2009; Loftsson and Masson, 2001; Nguyễn et al., 2017; Potier et al., 2012).

The discovery of deep eutectic solvents (DES) in 2003 was a turning point in the world of green chemistry (Abbott et al., 2003). Since then the publications in this field have increased exponentially. Being easy to prepare from cheap and widely available starting materials, DES have attracted a lot of attention from researchers in the past few years and have found applications in a wide range of domains: metal processing, synthesis, electrochemistry, solubilization of gas and pollutants, extraction of bioactive compounds and as drug solubilization vehicles (El Achkar et al., 2019; Li and Lee, 2016; Mbous et al., 2017; Morrison et al., 2009; Moura et al., 2017; Zhang et al., 2012). These solvents

65 are obtained by mixing two or three compounds that are able to associate, mainly by the
66 formation of hydrogen bonds, at a particular molar ratio to form a clear homogenous
67 liquid with a lower melting point than that of the individual components (Zhang et al.,
68 2012). Among them, DES based on carbohydrates and other primary metabolites have
69 been described lately (Choi et al., 2011). These DES are called natural deep eutectic
70 solvents (NADES). In this respect, cyclodextrins (CD) are potential NADES-forming
71 compounds.
72 Only a few studies have investigated the combined use of CD and DES. Some studies
73 have evaluated the possibility of forming low melting mixtures (LMM) with CD and *N*,*N*'-
74 dimethylurea (DMU) (Ferreira et al., 2015; Imperato et al., 2005; Jérôme et al., 2014). A
75 LMM was indeed obtained with various CD derivatives. Hydroformylation, Tsuji-Trost
76 and Diels–Alder reactions were efficiently performed in these new solvents with high
77 catalytic activity. However, the melting point of these LMM was superior to 80 °C,
78 limiting their scope of application. Thereafter, we have patented the use of DES for the
79 absorption of volatile organic compounds (VOC) and described the beneficial effect of
80 adding CD on VOC solubility in a choline chloride:urea (ChCl:U) DES (Fourmentin et al.,
81 2016). At the same time, a study showing that the solubility of the three native CD was
82 remarkably enhanced in the same DES was published (McCune et al., 2017). Moreover,
83 it was recently reported that no aggregation of β-CD occurred in ChCl:U up to
84 concentrations of 800 mg/mL (Triolo et al., 2020). Methyl-β-CD has also been
85 incorporated into aqueous solutions of a DES based on glycerol and glycine and
86 described to improve the extraction of polyphenols (Athanasiadis et al., 2018). Later, we
87 investigated the advantage of combining ChCl:U and CD to solubilize various volatile

compounds and demonstrated that CD maintain their host-guest properties in ChCl:U (Di Pietro et al., 2019; Dugoni et al., 2019; Moufawad et al., 2019). Combinations of DES and CD can potentially give rise to highly attractive materials; however, having a solvent based on CD could be even more useful. To this end, we recently reported the first supramolecular mixture based on randomly methylated-β-CD (RAMEB) and levulinic acid and showed that RAMEB retained its inclusion properties (El Achkar et al., 2020).

In the present study, three new supramolecular mixtures were prepared using new β-CD derivatives (HP-β-CD, CRYSMEB and Captisol®) in addition to RAMEB as hydrogen bond acceptors (HBA) with levulinic acid (Lev) as hydrogen bond donor (HBD) (Figure 1). Density, viscosity and rheological measurements, differential scanning calorimetry and thermal gravimetric analysis were carried out to characterize these new liquid mixtures. Moreover, their stability was evaluated as a function of time and temperature by NMR. Finally, their ability to solubilize trans-anethole (AN), star anise and fennel essential oils was evaluated by static headspace-gas chromatography (SH-GC) and multiple headspace extraction (MHE) was used to monitor the release of AN from these mixtures.

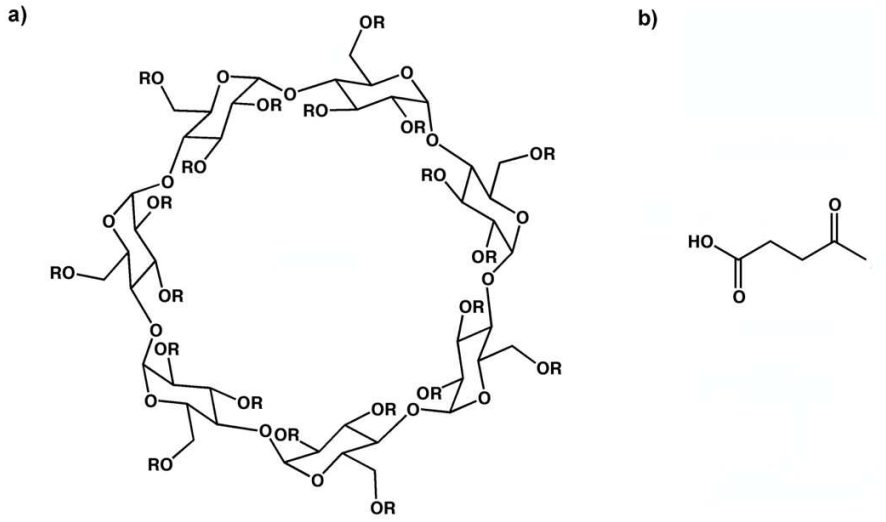


Figure 1. Structures of the low melting mixtures constituents a) General structure of β-cyclodextrin derivatives: HP-β-CD (degree of substitution (DS) = 5.6), R= -H or -CH_2-CH(OH)-CH_3; RAMEB (DS = 12.9), R= -H or -CH_3; CRYSMEB (DS = 4.9), R= -H or -CH_3; Captisol® (DS = 6.5), R= -H or -$(CH_2)_4$-SO_3^- Na^+; b) Levulinic acid.

2. Experimental Section

2.1. Materials

trans-Anethole [1-methoxy-4-(1-propenyl)-benzene] (99%) was purchased from Acros Organics, France. Levulinic acid (98%) was purchased from Sigma-Aldrich, France. Randomly methylated-β-CD (RAMEB, DS =12.9), low methylated-β-CD (CRYSMEB, DS = 4.9) and hydroxypropyl-β-CD (HPBCD, DS= 5.6) were provided by Roquette Frères (Lestrem, France). Sulfobutylether-β-CD (Captisol®, DS= 6.5) was provided by LIGAND Pharmaceuticals (San Diego, CA, USA). All compounds were used as received.

Essential oils of *Illicium verum* (star anise) and *Foeniculum vulgare* (fennel) were provided by Herbes et Traditions (Comines, France). The main constituents (>1%) of

123 the essential oils are: *trans*-anethole (91.07%) and limonene (3.28%) for star anise;
124 *trans*-anethole (75.30%), estragole (3.10%) and fenchone (2.10%) for fennel (data
125 provided by Herbes et Traditions).
126
127 *2.2. Preparation of the supramolecular mixtures*
128 The LMM were prepared by mixing the HBA and the HBD at a fixed molar ratio (Table
129 1). The mixtures were then stirred at 60 °C until the formation of a clear homogenous
130 liquid then cooled at room temperature. All the prepared mixtures were liquid at room
131 temperature. As CD possess a large number of HBA sites (35 sites for the native β-CD
132 and 35 to 55 sites for the modified β-CD), we used a large molar excess of levulinic
133 acid. The water content of all the prepared mixtures was determined using Karl Fisher
134 titration method (Mettler Toledo DL31) and was found to vary between 2.5 and 3.9 wt.%
135 (Table 1).
136
137 Table 1. Composition and water content of the prepared LLM.

CD:Lev mixture	**HBA**	**HBD**	**HBA:HBD molar ratio**	**Water content (wt. %)**
HPBCD:Lev	Hydroxypropyl-β-CD	Levulinic Acid	1:32	2.7
RAMEB:Lev	Randomly methylated β-CD		1:27	2.5
CRYSMEB:Lev	Low methylated β-CD		1:25	3.3
Captisol®:Lev	Sulfobutylether-β-CD		1:44	3.9

138
139 We should point out that our attempts to obtain liquid mixtures with the three native CDs
140 failed, which tends to emphasize that the density and flexibility of the hydrogen bond
141 network are probably key parameters for the formation of liquid mixtures. It seems that

142 the breakdown of hydrogen bonds from one glucose to another within CD molecules
143 could be a prerequisite, therefore limiting the preparation of such LMM to modified CDs.
144
145 *2.3. Stability studies of LMM by NMR*
146 In order to evaluate whether any esterification reaction occurred between the remaining
147 hydroxyl groups of the CD derivatives and levulinic acid, the supramolecular mixtures
148 were prepared at 30 °C and were subjected to gradual heating. Aliquots were withdrawn
149 at different times and temperatures, then diluted with DMSO-d_6, or $CDCl_3$, in the case of
150 Captisol®:Lev, and analyzed by NMR. The first aliquot of the mixture was taken when a
151 clear liquid was formed (t_0). The other aliquots were withdrawn at 30, 60, 80 and 100
152 °C. The mixtures were kept for 24 hours at the desired temperature prior to analysis. ^{13}C
153 NMR experiments were recorded on a Bruker Avance III spectrometer operating at 400
154 MHz for the proton nucleus, equipped with a multinuclear z-gradient BBFO probe head.
155 The probe temperature was maintained at 30 ˚C and standard 5 mm NMR tubes were
156 used. ^{13}C spectra were recorded with the following acquisition parameters: time domain
157 65 K with a digital resolution of 0.73 Hz, relaxation delay: 2 s and 1536 scans.
158
159 *2.4. Density and viscosity measurements*
160 Density measurements were carried out using a U-shaped vibrating-tube densimeter
161 (Anton Paar, model DMA 5000 M) operating in a static mode. The factory calibration
162 was used and verified before and after each measurement with air and tri-distilled
163 degassed water. The DMA 5000 densimeter performs an analysis with an estimated
164 uncertainty in density and temperature of ± 0.1 kg m^{-3} and ± 0.001 °C, respectively.

165 The viscosity was determined using a falling-ball-based microviscosimeter (Lovis 2000
166 M/ME from Anton Paar). The temperature was controlled to within 0.005 °C and
167 measured with an accuracy better than 0.02 °C. A capillary tube of 1.8 mm diameter,
168 previously calibrated as a function of temperature and angle of measurement with
169 reference oils, was used for the measurements. The overall uncertainty on the viscosity
170 was estimated to be 2%. All measurements were performed at atmospheric pressure
171 and at temperatures ranging between 30 and 60 °C, as previously described (Moufawad
172 et al., 2019).
173
174 *2.5. Rheological measurements*
175 Rheological measurements were performed with an AR-G2 controlled-stress rotational
176 rheometer (TA Instruments). Flow curves were obtained with an aluminum cone-plate
177 geometry (40 mm diameter, 1° cone angle, 28 µm truncation gap). A three-step shear
178 rate sweep was imposed after a 3-minute equilibration time: 1) increase of the shear
179 rate from 0.1 to 5000 s^{-1} over 3 min (upwards curve), 2) peak hold at 5000 s^{-1} during 1
180 min, 3) decrease of the shear rate from 5000 to 0.1 s^{-1} over 3 min (downwards curve).
181 The temperature was maintained at 30 °C and controlled with a Peltier plate.
182 Measurements were performed in triplicate at least for each sample, to ensure
183 reproducibility. The statistical analysis was performed by calculating the standard
184 deviation from the three measurements or more made for each sample.
185
186 *2.6. Differential scanning calorimetry (DSC)*

187 DSC experiments were carried out using a Q1000 DSC (TA Instruments) with a
188 temperature range from -100 °C to 40 °C and at a thermal scanning rate of 5 °C.min^{-1}.
189 All the samples (HPBCD:Lev, RAMEB:Lev, CRYSMEB:Lev and Captisol®:Lev) were
190 encapsulated in aluminum pans (sample weight ~ 10-15 mg), sealed with hermetic lids
191 and analyzed. Experiments were performed under nitrogen flow (50 mL.min^{-1}).
192
193 *2.7. Thermal gravimetric analysis (TGA)*
194 TG measurements were performed with a TGA550 thermogravimetric analyzer (TA
195 Instruments). Samples were placed in an open platinum pan (100 µL) suspended in the
196 furnace. The initial weight of the sample was around 25-30 mg, and nitrogen was used
197 as the purge gas at a fixed flow of 20 mL.min^{-1}. The weight of material was recorded
198 during heating from room temperature to 600 °C at a heating rate of 10 °C.min^{-1}.
199
200 *2.8. Static Headspace-Gas Chromatography (SH-GC)*
201 AN, star anise and fennel essential oils (EO) were added to water or to the LMM placed
202 in 22 mL headspace glass vials. Vials were then sealed and thermostated at 30 °C
203 under stirring for 24 hours in order to reach equilibrium between liquid and gaseous
204 phases. Subsequently, 1 mL of the gaseous phase was withdrawn from the vial and
205 injected in the chromatographic column for analysis via a heated transfer line.
206 All experiments were carried out with an Agilent G1888 headspace sampler coupled
207 with a Perkin Elmer Autosystem XL gas chromatography equipped with a flame
208 ionization detector and a DB624 column using nitrogen as carrier gas. The GC column
209 temperature was fixed at 160 °C for AN. For the analysis of EO, temperature conditions

were set as follows: initial temperature of 50 °C for 2 min, increased to 200 °C at 5 °C.min^{-1}, then hold for 2 min, giving a total runtime of 34 min.

2.8.1. Determination of partition coefficient (K)

The vapor-liquid partition coefficient (*K*) is the ratio of the concentration of a substance in vapor phase to its concentration in liquid phase, when the equilibrium is reached (Kolb and Ettre, 2006).

$$K = \frac{C_G}{C_L} \quad (1)$$

K of AN was determined in water and in the different supramolecular mixtures at 30 °C by using the phase ratio variation method for AN in water and the vapor phase calibration method for AN in the supramolecular mixtures as reported earlier (Moura et al., 2017).

2.8.2. *Retention of AN and essential oils (EO)*

The retention of AN, star anise and fennel EO in the formulations was estimated according to procedures developed for aromas (Decock et al., 2008) and EO (Kfoury et al., 2015), respectively. The percentage of retention of AN and EO by the LMM was determined by SH-GC at 30 °C following equation (2) for AN and equation (3) for EO:

$$\%\ Retention = \left(1 - \frac{A_{LMM}}{A_W}\right) \times 100 \quad (2)$$

where A_{LMM} and A_W stand for the peak area of AN in presence of LMM and of water, respectively.

$$\% \, Retention = \left(1 - \frac{\Sigma A_{LMM}}{\Sigma A_W}\right) \times 100 \quad (3)$$

where ΣA_{LMM} and ΣA_W stand for the sum of peak areas of the EO components in presence of LMM and of water, respectively.

2.8.3. Multiple Headspace Extraction (MHE)

MHE is a quantitative method, consisting of successive headspace extractions (Kolb and Ettre, 2006). It was used to study the release kinetics of AN from the prepared LMM at 60 °C. Samples were prepared similarly to the previous experiments but, once equilibrium was reached, vials were subjected to 10 headspace extractions at 1-hour intervals.

3. Results and Discussion

3.1. Stability studies of LMM by NMR

A recent study showed that ChCl:carboxylic acid DES underwent esterification regardless of the method or the temperature employed during their preparation. However, the DES based on levulinic acid was the least affected, with only 6 mol% of the ChCl esterified at 100 °C (Rodriguez Rodriguez et al., 2019). In the present study,

252 the thermal stability of the new supramolecular mixtures was investigated using ^{13}C
253 NMR, by following the modification of the chemical shift of the carboxylic acid function of
254 levulinic acid. All the supramolecular mixtures were stable after consecutive exposures
255 of 24 hours at 30 and 60 °C. After an additional 24 hours at 80 °C, a small amount of
256 esterification was observed and identified by the presence of a new broad signal around
257 173.0 ppm corresponding to the ester function (Figure S1). No esterification was
258 detected in the case of Captisol®:Lev, which could be due to steric hindrance from the
259 relatively bulky sulfobutyl ether moieties. On the other hand, the most affected LMM
260 was HPBCD:Lev with the relative intensity ratio of the esterified carbon peak being
261 1.6% of the carboxylic acid carbon peak at 100 °C (Table S1) and could be the result of
262 a higher reactivity of the hydroxyl groups, especially that of the hydroxypropyl moieties.
263 Therefore, the preparation of the LMM was performed at 60 °C for all other experiments,
264 in order to avoid esterification. Furthermore, the long-term stability of the LMM was
265 evaluated after 18 months of storage at room temperature. The mixtures showed no
266 degradation over time. The low esterification ratio observed for these CD-based
267 mixtures compared with ChCl-based DES could be explained by the fact that the
268 remaining hydroxyl groups of the CD derivatives used in this study are sterically
269 hindered since the degree of substitution varied from 4.9 to 12.9.
270
271 *3.2. Physicochemical characterization*
272 The density and viscosity values of the four LMM (HPBCD:Lev, RAMEB:Lev,
273 CRYSMEB:Lev and Captisol®:Lev) were measured from 30 to 60 °C, at 10 °C intervals
274 (Figure 2 and Tables S2 and S3). CD-based mixtures presented higher density values

(ranging between 1184.5 and 1234.3 kg m^{-3} at 30 °C) compared with common DES based on levulinic acid (1134.5 and 1105.7 kg m^{-3} at 303.15 K for ChCl:Lev or TBPBr:Lev DES respectively) (Moufawad et al., 2019; Moura et al., 2017). Additionally, the obtained values fall in the 1000-1300 kg m^{-3} range of density values observed with most of the reported DES (Tang and Row, 2013). LMM were also more viscous than these common DES, except for RAMEB:Lev which presents similar viscosity to ChCl:Lev (212.9 and 206.2 mPa.s at 30 °C, respectively).

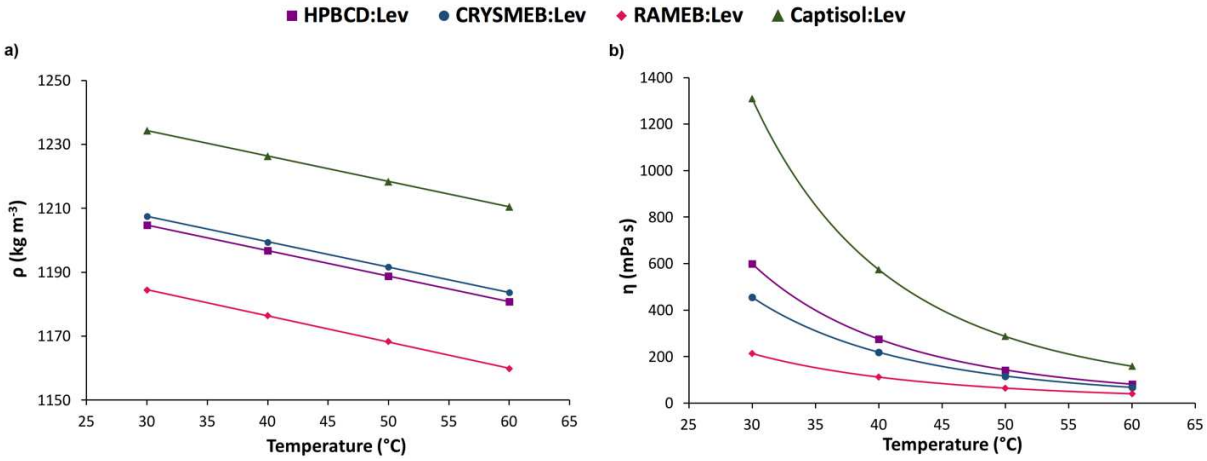


Figure 2. Experimental values of the density (a) and viscosity (b) of the supramolecular LMM. The lines represent the Vogel–Fulcher–Tammann (VFT) correlation fitting for viscosity and appropriate polynomials in the case of the density.

Nevertheless, the viscosity values decreased remarkably with increasing temperature. All CD-based mixtures, except Captisol®:Lev, show relatively low viscosities (≤ 80 mPa.s) at 60 °C. The relatively higher viscosity of Captisol®:Lev can be explained by a stronger hydrogen bond network, given the higher number of HBA sites present in Captisol® compared to the other studied β-CD derivatives due to the presence of the

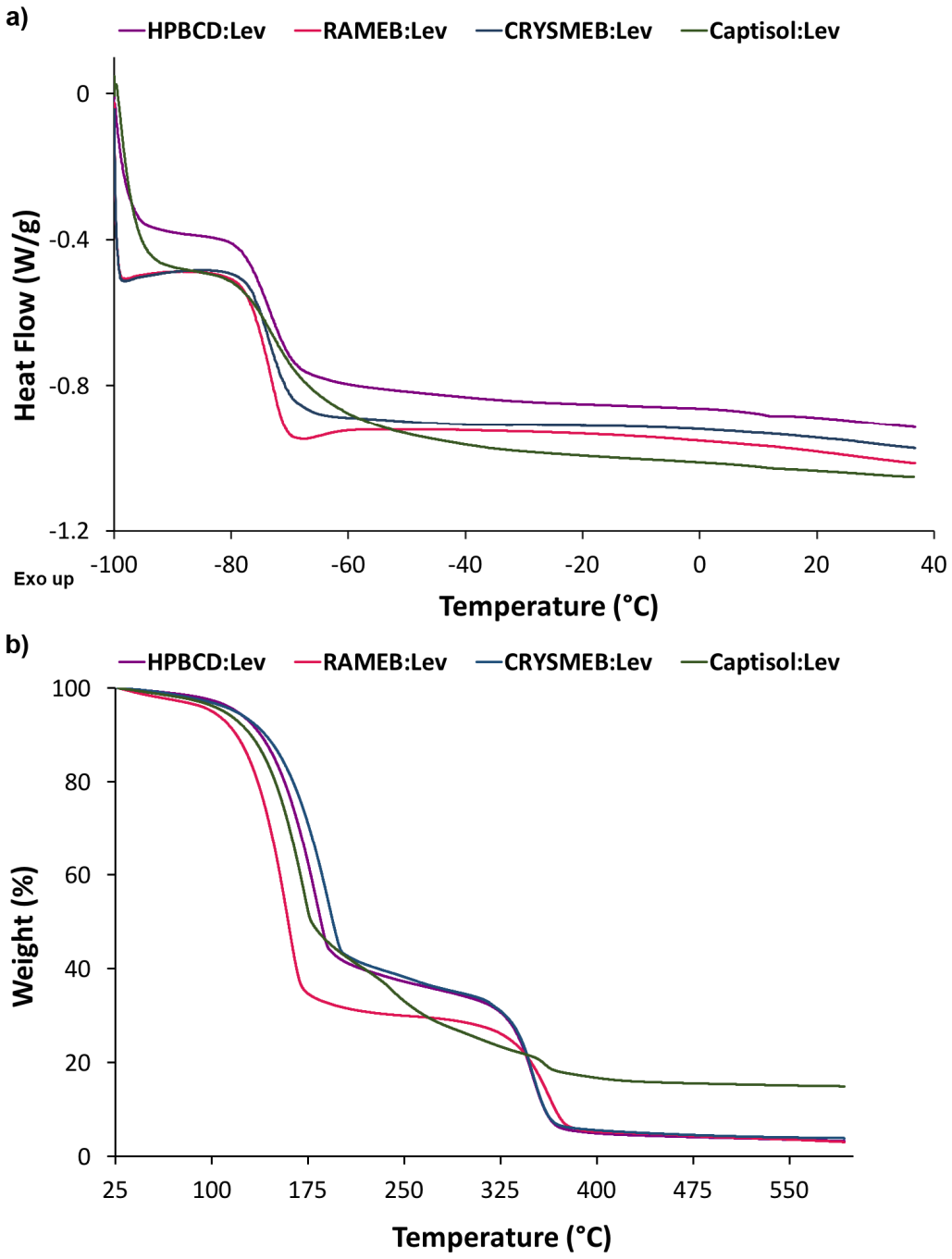
sulfonate groups. In addition, the CD:Lev mixtures were less viscous than the mixtures based on β-CD derivatives and *N,N'*-dimethylurea. Indeed, the latter mixtures present a melting point above 80 °C and their viscosities were at least equal to 205.0 mPa.s at 90 °C (Jérôme et al., 2014).

The flow behavior of the four LMM was then investigated at 30 °C. All the mixtures studied exhibit a Newtonian plateau for shear rates below 1000 s^{-1} and shear-thinning behavior for shear rates above this value (Figure S2). The measured static and dynamic viscosities for the same composition were slightly different (for example, in the case of RAMEB:Lev at 30 °C, η_{static} = 212.9 mPa.s vs. $\eta_{dynamic}$ = 245.0 mPa.s) but the same order was found for the viscosities ($\eta_{Captisol®:Lev} > \eta_{HPBCD:Lev} > \eta_{CRYSMEB:Lev} > \eta_{RAMEB:Lev}$). Moreover, the shear-thinning behavior was more pronounced as the viscosity increased. Indeed, for a high shear rate of 5000 s^{-1}, a decrease of around 29% in viscosity was observed for Captisol®:Lev, whereas in the case of RAMEB:Lev a smaller decrease (around 8%) was detected. As explained above, the most viscous mixtures probably contain a larger number of hydrogen bonds that may be disrupted at high shear rates, leading to a greater decrease in viscosity.

3.3. Thermal properties

DSC measurements were performed over a range of -100 to 40 °C in order to understand the thermal events occurring in this temperature range. We chose to focus on the lower temperature range, given that the samples under study were all liquid at room temperature. Interestingly, none of the CD-based mixtures, *i.e.* HPBCD:Lev, RAMEB:Lev, CRYSMEB:Lev and Captisol®:Lev, showed any melting point in the

316 heating curve. Instead, glass transition curves with T_g at -73.3, -74.3, -73.5 and -67.8 °C
317 were observed respectively for the mixtures (Figure 3a). This absence of melting peaks
318 and presence of glass transition temperature was widely observed in the literature. For
319 example, Francisco et al. reported 21 systems showing no melting point but glass
320 transition temperatures ranging between -13.64 and -77.73 °C (Francisco et al., 2012)
321 while Dai et al. identified 13 different NADES with T_g < -50 °C and no melting point (Dai
322 et al., 2013). Likewise, with the exception of glass transition, no other thermal events
323 occur for trehalose:glycerol at a 1:30 molar ratio (Castro et al., 2018). Moreover, the
324 observed T_g value of -75.14 °C is interestingly pretty close to the values obtained for our
325 CD-based mixtures. CD generally present a T_g ranging between 80 °C and more than
326 200°C depending on the CD (Tabary et al., 2011). Therefore, the observed T_g values
327 could be attributed to the formation of a LMM with levulinic acid.
328



329

330 Figure 3. DSC (a) and thermogravimetric analysis (b) curves of the LMM.

331

332 Dynamic thermogravimetric analysis (TGA) was used to further investigate the thermal

333 stability of the new mixtures. As shown in Figure 3b, they underwent progressive

decomposition as the temperature increased. Their decomposition showed a two-step weight loss similar to choline-based DES (Delgado-Mellado et al., 2018). At around 130 °C, levulinic acid began to decompose while the CD began to degrade at about 325 °C, except in the case of Captisol® for which the second decomposition began at around 225 °C and a third decomposition was determined near 350 °C. From the thermogravimetric curve in Figure 3b, the results showed that the thermal decomposition temperatures corresponding to the first thermal event of HPBCD:Lev, RAMEB:Lev, CRYSMEB:Lev and Captisol®:Lev were equal to 130.4, 117.6, 137.7 and 127.6 °C respectively. CRYSMEB:Lev had the best thermal stability and RAMEB:Lev the worst (Table S3). The DSC and TGA results demonstrate that these CD-based mixtures retain a stable liquid state over a wide temperature range.

3.4. Solubilization of AN and essential oils

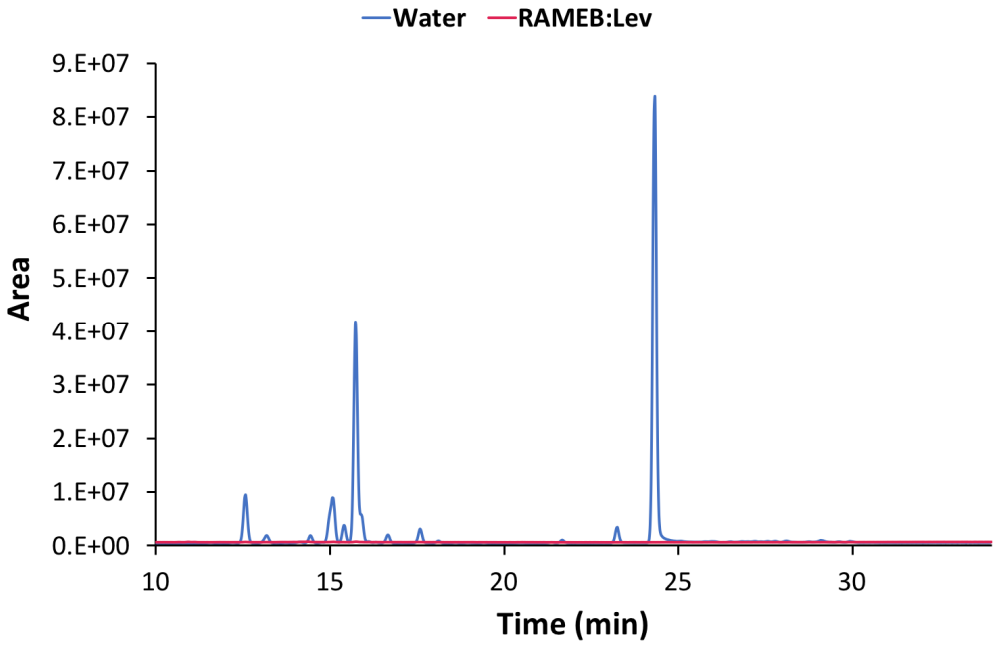
AN has incited interest from the food, cosmetics and pharmaceutical industries due to its attractive properties. In fact, AN is a major component of star anise and fennel essential oils which are known both as flavoring agents and as medicines owing to their numerous biological properties (Auezova et al., 2020; Diao et al., 2014; Wang et al., 2018). However, its wider applications are hampered by drawbacks related to its high volatility, low water solubility and chemical instability. We have previously investigated the effect of AN complexation with various CD on its solubility and photodegradation and showed that CD were able to improve AN solubility and stability through inclusion complex formation (Kfoury et al., 2014a, 2014b). Therefore, the ability of the LMM to solubilize AN was evaluated by determining the vapor-liquid partition coefficient *K* of AN

357 (Table 2) as well as the percentage of AN retention. More than 99% of AN were
358 retained by all the studied mixtures while in aqueous solution CD were able to reduce
359 AN volatility only up to 92% leading to around 20-fold reduction in *K* (Kfoury et al.,
360 2014b, 2014a). In the case of the supramolecular mixtures we observed up to 1300
361 times reduction in *K* compared with water (Table 2).
362
363 Table 2. Partition coefficient (*K*) values of *trans*-anethole in water and in the tested
364 mixtures and ratio of K_{water}/K_{LMM} at 30°C
365

	K	Ratio
Water	1.29×10^{-2}	1
HPBCD:Lev	1.04×10^{-5}	1240
RAMEB:Lev	9.60×10^{-6}	1343
CRYSMEB:Lev	1.08×10^{-5}	1194
Captisol®:Lev	2.18×10^{-5}	592

366
367 DES have been explored as solvents for the extraction of EO or their components in the
368 past few years (Li et al., 2019; Ozturk et al., 2018), with higher extraction yields
369 observed compared with conventional solvents. However, the majority of the reported
370 studies used diluted DES to improve their extraction efficiency by reducing their
371 viscosity or increasing their polarity (Jeong et al., 2018). To the best of our knowledge,
372 no studies investigating the solubilization or the extraction of AN or star anise EO using
373 DES have been performed to date. Only one publication has reported the use of lactic
374 acid-based NADES for the extraction of polyphenols from fennel EO, among other

375 Greek medicinal plants (Bakirtzi et al., 2016). Subsequently, the ability of the studied
376 LMM to solubilize EO, as well as AN in the presence of other aroma compounds, in two
377 EO was investigated.
378 Star anise and fennel EO were chosen for having AN as a major component. All the
379 tested mixtures showed high retention ability (≥ 98.9%) toward star anise and fennel EO
380 (Figure S3). Figure 4 depicts the chromatogram of star anise EO obtained by SH-GC in
381 presence of RAMEB:Lev and in presence of water as a reference solvent. Clearly, the
382 peaks corresponding to the volatile compounds found in star anise are obvious in water
383 but almost disappear in presence of the CD-based mixture. Figure S4 shows the
384 chromatograms of star anise EO in the three other mixtures.
385



386
387 Figure 4. Chromatogram of star anise essential oil in water and in RAMEB:Lev.
388

Concerning the retention of AN, similar results were obtained regardless of whether AN was added in pure form or as a constituent of star anise or fennel EO (Figure S5). Therefore, the ability of the mixtures to retain AN was not affected by the simultaneous presence of other compounds.

3.5. Release study

Finally, the release of AN from the LMM was monitored by MHE. As described above, 10 successive headspace extractions were conducted at one-hour intervals. The amount of AN present in the vial after each extraction could be determined using the peak area. The retention ability of the LMM can be monitored and compared to water. The release profiles of AN from LMM and water are shown in Fig. 5. The results indicate that these CD-based mixtures can not only solubilize AN but also delay its release over time. Indeed, 70% of AN was released from water after 10 extractions at 60 °C, while only 20-40% was released from the CD:Lev mixtures.

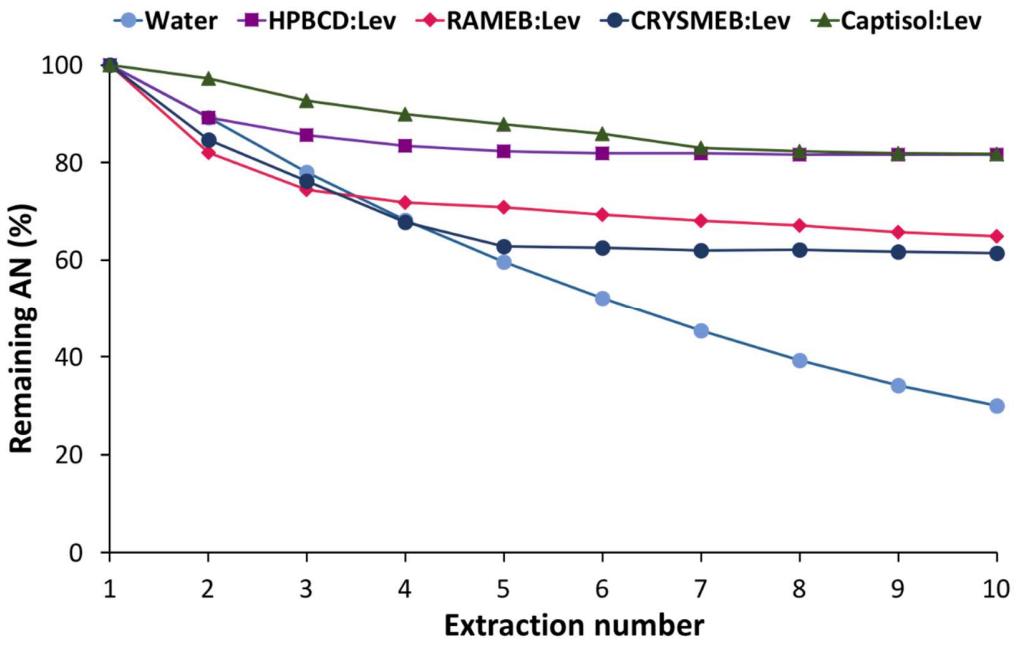


Figure 5. Release of *trans*-anethole from water and from supramolecular mixtures.

4. Conclusions

New supramolecular mixtures based on β-CD derivatives and levulinic acid were prepared, characterized and studied for their ability to solubilize AN and related essential oils. In addition, their thermal stability was investigated. The physicochemical characterization studies demonstrated that these CD-based mixtures are liquid at room temperature with relatively low viscosity. The stability studies also revealed that they were stable up to 60 °C and that no degradation could be detected after 18 months of storage. These mixtures also presented high solubilizing ability towards AN, star anise and fennel essential oils, when compared with water or aqueous solutions of CD. Furthermore, they were able to delay the release of AN, as shown by MHE experiments. When compared to aqueous CD solutions, these mixtures guarantee the presence of high amounts of CD associated to a liquid state over a wide temperature range, which consequently allow the broadening of CD applications. These mixtures may therefore contribute in the search for greener solvents and result in new promising, safe and economic drug delivery systems. Indeed, due to their peculiar properties (viscosity, stability and encapsulation properties), these supramolecular LMM could solubilize poorly water-soluble drugs and/or protect fragile molecules during topical cutaneous administration. The potential of these LMM is currently being tested for the treatment of cutaneous leishmaniasis with a volatile and poorly water-soluble anti leishmaniasis drug and the results will be communicated in due course.

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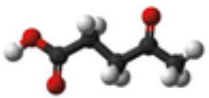
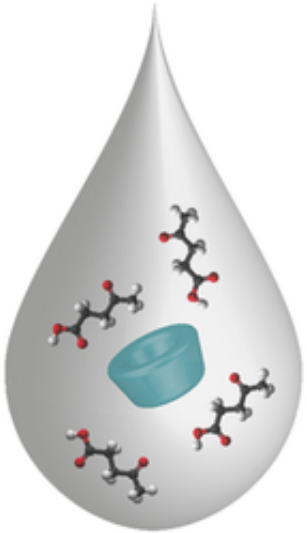
Modified β-cyclodextrins

mp > 280 °C

+

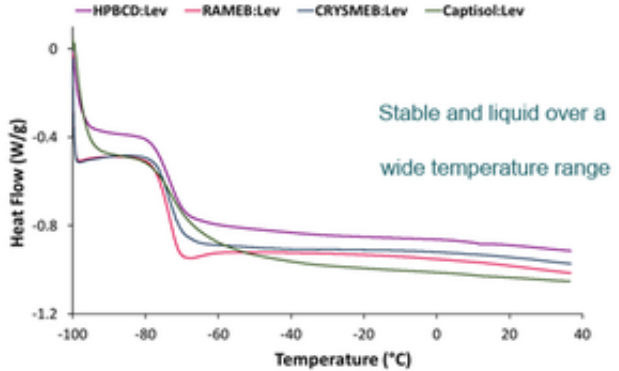


Levulinic acid



New liquid mixtures based on cyclodextrins

Physicochemical characterization



Solubilization of bioactive compounds